A novel approach using the 15N tracer technique and benthic chambers to determine ammonium fluxes at the sediment-water interface and its application in a back-reef zone on Reunion Island (Indian Ocean)

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A novel approach using the ^{15}N tracer technique and benthic chambers to determine ammonium fluxes at the sediment–water interface and its application in a back-reef zone on Reunion Island (Indian Ocean)

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A B S T R A C T

The ^{15}N tracer method and the benthic chamber technique were combined to evaluate NH_4^+ exchanges at the sediment–water interface. This novel approach consists in measuring NH_4^+ fluxes during a single in situ incubation in a sample of water enclosed in a benthic chamber placed over the sediment and in a subsample thereof concomitantly incubated in a bottle. Using this combined approach, the influx and efflux of NH_4^+ across the sediment–water interface can be simultaneously measured along with uptake and regeneration rates of NH_4^+ in the water column. Details of the experimental protocol and principles behind the calculations of N transport rates are given. We applied this approach to a tropical reef on Reunion Island (Indian Ocean). Experiments were carried out in triplicate at three stations with organic-poor, sandy sediments. At the three stations, the mean flux of NH_4^+ from the water column to the sediment (29.6–59.2 μmol m^{-2} h^{-1}) was much higher than the mean NH_4^+ uptake rate by phytoplankton (3.0–4.0 μmol m^{-2} h^{-1}) indicating that the removal of NH_4^+ from the water column must be due, for the most part, to uptake by benthic microalgae in the study area. The mean flux of NH_4^+ from the sediment to the water column (6.7–13.7 μmol m^{-2} h^{-1}) was comparable to the mean regeneration rate in the water (7.4–9.9 μmol m^{-2} h^{-1}) suggesting that the sediment may constitute a significant N source for phytoplankton in the back-reef zone on Reunion Island.

1. Introduction

Exchanges between the pelagic and benthic compartments play an important role in the coastal nitrogen (N) cycle. For instance, in bottom sediment, mineralisation of particulate organic matter resulting from pelagic and benthic production leads to the formation of dissolved inorganic nitrogen (DIN), in particular ammonium (NH_4^+), which can then be transferred to the water column. The role of DIN supply in pelagic primary production is well known in shallow coastal environments (Anderson et al., 2003; Cowan and Boynton, 1996; Harrison, 1980; Joye and Anderson, 2008; Nixon, 1981; Rowe et al., 1975). Various processes can prevent NH_4^+ transfer to the water column. A fraction of regenerated NH_4^+ may be adsorbed onto particles through association with ion-exchange sites (Burdige, 2006; Klump and Martens, 1983), although adsorption is a reversible process that depends for the most part on redox conditions (Rosenfeld, 1979). Substantial removal of NH_4^+ from sediments can also occur through oxidation to nitrite (NO_2^-) and then nitrate (NO_3^-) by nitrifying microorganisms (Capone et al., 1992; Mortimer et al., 2004; Thamdrup and Fleischer, 1998), or through anaerobic ammonium oxidation to molecular nitrogen (N_2) (Dalsgaard and Thamdrup, 2002; Engstrom et al., 2005; Risgaard-Petersen et al., 2004). In shallow-water with well-lit sediment surfaces, the use of sedimentary NH_4^+ as a source of N for autotrophic benthic organisms at the sediment–water interface can also restrict NH_4^+ release into the water column (Anderson et al., 2003; Bartoli et al., 2003; Eyre and Ferguson, 2005; Lomstein et al., 1998; McGlathery et al., 2001; Sundbäck et al., 2000; Veuger et al., 2007). In well-lit conditions, NH_4^+ can be taken up from the water column if sediment DIN sources are insufficient to meet the growth demands of the benthic community (Joye and Anderson, 2008). NH_4^+ exchanges between the benthic compartment and the water column thus strongly depend on benthic algae biomass and light availability. Although the production of NH_4^+ resulting from the degradation of organic matter likely continues in the light, the removal of NH_4^+ from the water column and the decrease in sedimentary NH_4^+ supply due to consumption by benthic algae can lead to a net influx of NH_4^+ into the sediment (Joye and Anderson, 2008; Veuger et al., 2007).

A number of approaches have been used to study NH_4^+ fluxes at the sediment–water interface. Rates of NH_4^+ release from sediments were first studied using a geochemical approach in which the estimated flux is based on the vertical profiles of NH_4^+ concentrations in interstitial

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waters (Berner, 1974, 1977; Vanderborght et al., 1977). However, in coastal sediments, the horizontal and vertical gradients of NH_4^+ vary with time and benthic macrofauna activity (Kristensen, 1988). Another method consists in collecting sediment cores and monitoring the variation in NH_4^+ concentrations in the overlying water over time (e.g. Blackburn et al., 1988; Seitzinger, 1987). One disadvantage of this method is that the state of the sediment and its layered structure as well as the chemical characteristics of the surrounding water can be significantly altered during coring and sampling (Viollier et al., 2003). With the use of in situ benthic chambers, NH_4^+ fluxes between sediment and water at the sea floor can be measured directly. The changes in NH_4^+ concentration in the overlying water over time are used to calculate the exchanges at the sediment–water interface (e.g. Boucher and Clavier, 1990; Boucher et al., 1994; Clavier et al., 2005). Water column processes are considered negligible or are estimated during parallel incubations, for example, in benthic chambers with bottoms that exclude the sediment (Neubauer et al., 2005). However, benthic chamber techniques only provide the net fluxes of NH_4^+, i.e. the difference between the release from the sediment and the removal from the water column. Light and dark incubations are sometimes performed simultaneously to distinguish between the fluxes of NH_4^+ into and out the sediment (Joye and Anderson, 2008), but the two fluxes cannot always be separated because both microphytobenthos (MPB) and bacteria can assimilate N in the dark (Evrard et al., 2008). Another useful approach for studying NH_4^+ fluxes in the benthic and pelagic compartments is the ^{15}N tracer method. Since its introduction in the 1960s (Dugdale and Goering, 1967), the ^{15}N tracer method has been intensively used to measure NH_4^+ uptake by the microbial community in the water column. An isotope dilution technique using a ^{15}N tracer was later developed to measure NH_4^+ regeneration in the water column (Glibert et al., 1982). This isotope dilution method has also been applied to estimate the gross rate of NH_4^+ production in marine sediments with the addition of $^{15}NH_4^+$ in intact sediment cores or in the overlying water, as described first by Blackburn (1979) and modified by Anderson et al. (1997) and Jochem et al. (2004). Ammonium incorporation by the sediment is generally estimated in parallel, either indirectly from the change in the quantity and in the ^{15}N-content of the NH_4^+ pool (Blackburn, 1979) or directly from an increase in the ^{15}N-content of organic N (Iizumi et al., 1982). The ^{15}N tracer method has also been used to determine the relative contribution of bacteria and MPB to the total benthic microbial NH_4^+ uptake (Cook et al., 2007; Evrard et al., 2008; Veuger et al., 2005) and to examine the uptake and retention of NH_4^+ by macroalgae and seagrasses (Dudley et al., 2001; Lepoint et al., 2004; Vonk et al., 2008). Nevertheless, the use of ^{15}N tracers is particularly rare in studies integrating both the pelagic and benthic compartments (Iizumi et al., 1982; Lepoint et al., 2004) and the compartments are either studied separately (Iizumi et al., 1982) or the studies do not account for the release of NH_4^+ from the sediment and its regeneration in the water column (Lepoint et al., 2004).

Here, we present a novel approach, combining the ^{15}N tracer method and the benthic chamber technique, to study NH_4^+ exchanges at the sediment–water interface. Our method simultaneously measures the fluxes of NH_4^+ into and from the sediment as well as uptake and regeneration rates of NH_4^+ in the water column. We applied this method to a tropical reef on Reunion Island (Indian Ocean).

2. Materials and methods

2.1. Method for measuring NH_4^+ fluxes at the sediment–water interface

The method consists in measuring, with a ^{15}N tracer, NH_4^+ fluxes during a single in situ incubation in a sample of water enclosed in a benthic chamber placed over the sediment and in a subsample thereof concomitantly incubated in a bottle. The benthic chamber experiment measures total NH_4^+ production, including NH_4^+ regeneration by microheterotrophs in the water column and NH_4^+ transfer from sediment to the water column and estimates NH_4^+ uptake by phytoplankton. The concomitant bottle experiment measures NH_4^+ regeneration by microheterotrophs in the water column alone, along with NH_4^+ uptake by phytoplankton. The efflux of NH_4^+ across the sediment–water interface can be determined by subtracting the NH_4^+ regeneration by microheterotrophs measured in the bottle from the total NH_4^+ production measured in the benthic chamber. The influx of NH_4^+ from the water column to the sediment can be deduced by taking into account the total NH_4^+ production, the NH_4^+ loss due to phytoplankton uptake and the variation in NH_4^+ concentration during the incubation period in the benthic chamber. Ammonium uptake rates were estimated by the incorporation of ^{15}N-labelled NH_4^+ into particulate organic nitrogen (PON) (Dugdale and Goering, 1967) and NH_4^+ production rates using the isotope dilution method (Glibert et al., 1982).

2.2. Study area

Experiments were carried out on the tropical island of Reunion (Indian Ocean). The study site was located in the back-reef zone of the Saint Gilles-La Saline fringing reef, the largest reef on Reunion Island (9 km long; maximal width 500 m). The experimental area features depths varying from 1.0 to 1.5 m, depending on the tidal level, and is protected from ocean waves by the reef flat. Experiments were carried out around noon local time in triplicate at three stations located in the same sector of the back-reef zone, at about 50 m downshore from the beach (Fig. 1). At all stations, the sediment was composed of sand scattered with coral fragments, covering a bed of limestone. All the experiments were carried out in calm weather, thereby minimising hydrodynamic forcing on the sediment surface.

2.3. Experimental protocol

The NH_4^+ fluxes at the water–sediment interface were assessed using three benthic chambers made of 0.2 m^2 cylindrical PVC tubes (Clavier et al., 2008). They were inserted into the sediment down to the underlying hard limestone substrate (ca. 10 cm) and covered with clear acrylic domes. The total volume varied from 47 to 63 L, depending upon the depth of insertion. The enclosed water was homogenised by a pump adjusted to a fixed flow rate of 2 L min^{-1}, which was the minimum value to ensure good mixing of water in the chambers. Multiparameter probes (YSI 6920) measured depth, temperature and salinity in the recirculating flow system. The values were recorded every second and averaged per minute.

The deployment of benthic chambers and sampling were carried out by scuba diving. Water samples were collected within the chambers using a polyethylene syringe (0.6 L) or using the recirculating flow system of the benthic chambers and polycarbonate flasks (2 L) (^{15}N bottle experiments). The benthic chambers, the recirculating flow system and the sampling equipment (syringe, flask) were washed with HCl (10%) and rinsed with deionised water.

Following deployment of benthic chambers, water samples (0.6 L) were immediately collected from inside each chamber to determine NH_4^+ and chlorophyll *a* (Chl *a*) concentrations. Chl *a* samples were filtered on 25-mm-Whatman-GF/F-filters. The filters were stored at −20 °C and analysed at the end of the field work. Ammonium concentrations were determined in triplicate on the filtrates recovered after the filtration of the Chl *a* samples. The ^{15}N tracer, in the form of $^{15}NH_4Cl$ (99% at %^{15}N, CEA, France), was then inoculated at a concentration of about 1 μmol N L^{-1} in the benthic chambers with a syringe (0.1 L) handled on-site by a scuba diver. After approximately 30 min, estimated as the time required to ensure homogenisation of the tracer, 1 L was sampled from the benthic chambers and filtered onto 25-mm-precombusted-Whatman-GF/F-filters under a vacuum of <100 mm Hg to determine the time-zero concentration and ^{15}N enrichment of the particulate and dissolved N pools. Filters were oven-dried at 60 °C for 24 h and stored until PON and isotopic analyses. A fraction of the filtrate

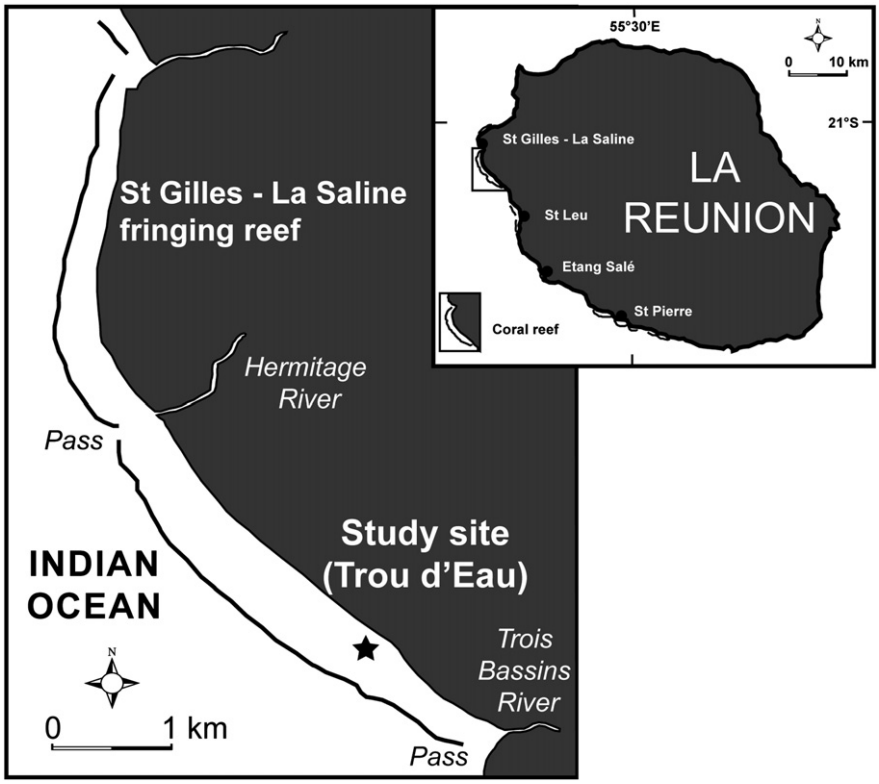

Fig. 1. Location of the study site on Reunion Island in a back-reef zone.

(about 0.4 L) was used immediately for triplicate NH_4^+ measurements and the remaining stored with $HgCl_2$ (20 μg mL^{-1}) pending measurements of ^{15}N enrichment in the NH_4^+ fraction. Outside water was allowed to enter through a tap during sampling to avoid interstitial water release from the sediment. This input caused a dilution of the NH_4^+ concentration and $^{15}NH_4^+$ enrichment. The initial enrichment of the NH_4^+ pool, given this dilution, varied from 51 to 71 atom% excess of ^{15}N in the benthic chambers. At the end of the incubation period (about 4 h), a sample from each chamber (1 L) was filtered onto 25 mm pre-combusted Whatman-GF/F-filters. The filters and the filtrates were treated as for the time-zero filtration.

Samples for ^{15}N bottle experiments were collected from within each chamber just after the sampling of water for the determination of NH_4^+ and Chl *a* concentrations. The ^{15}N tracer was added to the bottles on the beach at the same concentration (1 μmol N L^{-1}) as in the benthic chamber. The samples in bottles were separated into two aliquots immediately after the addition of the tracer. The first aliquot (1 L) was used to determine the time-zero concentration and ^{15}N enrichment of the particulate and dissolved N pools as described above. The initial enrichment of the NH_4^+ pool ranged from 61 to 69 atom% excess of ^{15}N. The second aliquot was incubated in 1 L polycarbonate bottles near the benthic chambers under in situ conditions. It was recovered at the end of the incubation period along with samples of benthic chambers and was treated in the same way.

At the end of each set of experiments, three sediment cores (Ø 35 mm) were sampled by hand within each chamber to determine the N content and ^{15}N content in excess in the sediment. The 0–0.5 cm and 0.5–1 cm layers were cut, placed in petri dishes, frozen and stored until analysis. Three sediment cores were also sampled outside the chambers to assess natural ^{15}N enrichment in the top centimetre of sediment. Based on these measurements, together with those done in the water column, we were able to carry out a ^{15}N inventory for each experiment.

2.4. Biological, chemical, and isotopic analyses

Chlorophyll *a* concentrations were measured fluorometrically (Yentsch and Menzel, 1963) in a Turner Designs fluorometer with a precision (SD) of ±0.05 μg L^{-1}. Ammonium concentrations were measured manually by the indo-phenol blue method (Koroleff, 1970) with a precision (SD) of ±10 nmol L^{-1}.

For isotopic analyses, NH_4^+ was first extracted from the filtrate by diffusion in basic pH (Kristiansen and Paasche, 1989). To 200 mL of filtrate placed in a 500 mL Erlenmeyer flask, we added 100 mg of MgO to raise the pH (>9) and 2 μmol N of unlabelled NH_4^+ (as a carrier). A strip of pre-combusted GF/C filter wetted with 50 μL of 0.5 N H_2SO_4 was suspended above the sample. The flask was capped tightly and left for 24 h at 60 °C in an oven. The strip was then removed and stored until isotopic analyses. Sediment samples were dried (60 °C for 24 h) and ground before analysis.

PON concentration in the water, N content in the sediment and ^{15}N enrichments of the PON, the sediment and the NH_4^+ recovered from the filtrate were quantified with a mass spectrometer (Delta plus, ThermoFisher Scientific, Bremen, Germany) coupled with a C/N analyser (Flash EA, ThermoFisher Scientific) via a type III-interface. The standard deviations (SD) were 0.004 μmol L^{-1}, 0.2 μmol N g dry sed^{-1} and 0.0001 atom% for PON concentration, N content and ^{15}N enrichment, respectively.

2.5. Calculation of nitrogen fluxes

Ammonium uptake rates (μmol L^{-1} h^{-1}) in bottles ($\rho_{W\text{-}BOT}$) and in benthic chambers ($\rho_{W\text{-}BEN}$) were calculated using the equation given by Dugdale and Wilkerson (1986) where the PON concentration is measured at the end of the incubation. Uptake rates were corrected for isotope dilution due to the production of $^{14}NH_4^+$ during the incubation which dilutes the $^{15}NH_4^+$ added at the start of the experiment

(Glibert et al., 1982); the correction factor varied from 1.0 to 1.1 (average: 1.05). The large amount of ^{15}N tracer added significantly increased the ambient NH_4^+ concentration and, consequently, probably also NH_4^+ uptake by phytoplankton, so, the uptake values given may overestimate the actual uptake rates (Dugdale and Wilkerson, 1986). Ammonium regeneration rates in bottles ($R_{W\text{-}BOT}$) and NH_4^+ production rates in benthic chambers (R_{BEN}) (μmol L^{-1} h^{-1}) were calculated from the Laws (1984) equation or that of Glibert et al. (1982), depending upon whether there were measurable changes in NH_4^+ concentration over the course of the experiment or not. Ammonium uptake and regeneration rates were integrated over the water column (μmol m^{-2} h^{-1}) by assuming that there was no vertical variation.

Ammonium production measured in benthic chambers (R_{BEN}) included regeneration in the water by microheterotrophs ($R_{W\text{-}BEN}$) and transport of NH_4^+ from the sediment to the water column (F_{SW}):

$$R_{BEN} = R_{W\text{-}BEN} + F_{SW}. \quad (1)$$

Ammonium regeneration in the water by microheterotrophs was necessarily the same in the benthic chambers ($R_{W\text{-}BEN}$) and in the bottles ($R_{W\text{-}BOT}$), because the two incubation systems contained the same water. Ammonium flux from the sediment to the water column can then be calculated using the following equation:

$$F_{SW} = R_{BEN} - R_{W\text{-}BOT}. \quad (2)$$

Ammonium transfer from the water column to the sediment (F_{WS}) was determined by taking into account the variation in concentration and the fluxes measured inside the benthic chambers. The variation in NH_4^+ concentration (ΔNH_4^+) resulted from the different fluxes in water ($\rho_{W\text{-}BEN}$ and $R_{W\text{-}BEN}$) and sediment–water interface (F_{SW} and F_{WS}):

$$\Delta NH_4^+ = (R_{W\text{-}BEN} + F_{SW} - \rho_{W\text{-}BEN} - F_{WS}) \times \Delta T \quad (3)$$

where ΔT is the length of the incubation period (h).

The variation in NH_4^+ concentration (ΔNH_4^+), the NH_4^+ production ($R_{BEN} = R_{W\text{-}BEN} + F_{SW}$) and the NH_4^+ loss due to phytoplankton uptake ($\rho_{W\text{-}BEN}$) were directly measured in the benthic chambers. The transfer from the water column to the sediment (F_{WS}) can then be calculated using the following equation:

$$F_{WS} = (R_{W\text{-}BEN} + F_{SW} - \rho_{W\text{-}BEN}) - \Delta NH_4^+/\Delta T. \quad (4)$$

The fluxes F_{SW} and F_{WS} represent the quantities of NH_4^+ exchanged between the water column and the sediment per litre of sea water enclosed in the benthic chamber and are expressed in μmol L^{-1} h^{-1}. They were converted into μmol m^{-2} h^{-1} based on the volume of the chamber and its exchange surface with the sediment (0.2 m^2).

To determine the mass balance of the ^{15}N tracer in each bottle and chamber, we calculated the ^{15}N content in excess (in μmol L^{-1} for bottles and in μmol for chambers) in the dissolved and particulate pools of the water column and in the sediment by multiplying the ^{15}N atom% excess enrichment by the nitrogen content. The amount of ^{15}N tracer in excess in the sediment was estimated for each chamber by taking the mean of the triplicate measurements in the 0–0.5 and 0.5–1 cm surface layers. The ^{15}N in excess recovered at the end of the incubation period was compared to the ^{15}N in excess added at the beginning of the experiment.

Paired-sample Wilcoxon signed-rank tests were used to test whether the differences observed during the experiments were statistically significant ($p < 0.05$). Non-parametric tests were used due to the small number of replicates and their non-normal distribution.

3. Results

3.1. Environmental parameters

The physical, chemical and biological characteristics of the water enclosed in the chambers are presented in Table 1. The initial temperature and salinity in the water were, respectively, about 25 °C and 35 whatever the station. There were no significant variations in temperature (Wilcoxon signed-rank test, $n = 9$, $p > 0.05$) or salinity (Wilcoxon signed-rank test, $n = 6$, $p > 0.05$) during the incubations, indicating that environmental conditions were stable in the chambers during the experiments. The mean NH_4^+ concentration varied from 0.30 to 0.37 μmol L^{-1} depending on the station. The mean Chl *a* and PON concentrations ranged, respectively, from 0.11 to 0.13 μg L^{-1} and from 0.25 to 0.30 μmol L^{-1}. PON concentrations did not vary significantly (Wilcoxon signed-rank test, $n = 9$, $p > 0.05$) within chambers between the beginning and the end of the incubation period, indicating that the flow rate of the recirculating flow system was high enough to prevent sedimentation of particles, but low enough not to resuspend the sediment. All the parameters were very similar in the three chambers at each station (coefficient of variation (CV) ≤ 10%) and varied little among stations (CV ≤ 17%) (Table 1).

3.2. Nitrogen flux

Mean NH_4^+ uptake rates measured in bottles ranged from 2.7 to $3.3 \cdot 10^{-3}$ μmol L^{-1} h^{-1} depending on the station, with a mean CV for triplicate measurements of 14.3% (Table 2). Mean NH_4^+ regeneration rates from the bottle experiments ranged from 6.2 to 9.0 10^{-3} μmol L^{-1} h^{-1} with a mean CV of 17.8% (Table 2). Mean ammonium uptake and regeneration rates integrated over the water column varied from 3.0 ± 0.4 to 4.0 ± 0.7 μmol m^{-2} h^{-1} and from 7.4 ± 1.5 to 9.9 ±1.7 μmol m^{-2} h^{-1}, respectively (Fig. 2).

Mean NH_4^+ uptake rates measured in benthic chambers (2.9–5.1 · 10^{-3} μmol L^{-1} h^{-1}) were not significantly different from those observed in the bottle experiments (Wilcoxon signed-rank test, $n = 9$, $p > 0.05$) and the mean CV (15.8%) was comparable to that found in the bottle experiments (Table 2). NH_4^+ production rates, which included water regeneration by microheterotrophs and transport from the sediment, ranged on average from 35.9 to

Table 1
Environmental parameters (mean ± SD, n = 3) in the water enclosed in the chambers at the three sampling stations. The values of the parameters measured at the end of the incubation period are given in parentheses.

Date	Depth (m)	Temperature (°C)	Salinity	NH_4^+ (μmol L^{-1})	Chl *a* (μg L^{-1})	PON (μmol L^{-1})
01/09/2005	1.1	24.87 ± 0.06 (26.22 ± 0.2)	nd[a] nd[a]	0.30 ± 0.03	0.13 ± 0.01	0.30 ± 0.03 (0.26 ± 0.02)
02/09/2005	1.1	25.16 ± 0.10 (26.27 ± 0.09)	34.54 ± 0.45 (34.60 ± 0.46)	0.34 ± 0.01	0.11 ± 0.01	0.25 ± 0.02 (0.25 ± 0.01)
03/09/2005	1.2	24.98 ± 0.20 (26.28 ± 0.08)	35.07 ± 0.09 (35.14 ± 0.04)	0.37 ± 0.01	0.11 ± 0.01	0.25 ± 0.01 (0.25 ± 0.01)

[a] Not determined.

Table 2
Ammonium uptake and regeneration rates in bottles ($\rho_{W\text{-}BOT}$ and $R_{W\text{-}BOT}$) and NH_4^+ uptake and production rates in benthic chambers ($\rho_{W\text{-}BEN}$ and R_{BEN}), changes in NH_4^+ concentration during the incubation period (ΔNH_4^+) and NH_4^+ fluxes from the sediment to the water column (F_{sw}) and from the water column to the sediment (F_{ws}) for each replicate at the three sampling stations.

Date	Replicate	Vol. chamber[a] (L)	ΔT[b] (h)	$\rho_{W\text{-}BOT}$ (10^{-3} μmol L^{-1} h^{-1})	$R_{W\text{-}BOT}$ (10^{-3} μmol L^{-1} h^{-1})	$\rho_{W\text{-}BEN}$ (10^{-3} μmol L^{-1} h^{-1})	R_{BEN} (10^{-3} μmol L^{-1} h^{-1})	ΔNH_4^+ (μmol L^{-1})	F_{sw} (10^{-3} μmol L^{-1} h^{-1})	F_{ws} (10^{-3} μmol L^{-1} h^{-1})
01/09/2005	1	46.6	4.1	3.3	9.5	2.8	50.9	−0.39	41.4	143.2
	2	48.5	4.1	2.7	7.9	3.0	27.9	−0.39	20.0	120.0
	3	50.5	4.1	2.9	7.2	3.1	28.8	−0.32	21.6	103.7
			Mean	3.0	8.2	3.0	35.9	−0.37	27.7	122.3
			SD	0.3	1.2	0.2	13.0	0.04	11.9	19.9
			CV (%)	10.0	14.6	6.7	36.2	10.8	43.0	16.3
02/09/2005	1	62.5	4.1	2.3	9.9	1.8	63.4	−0.46	53.5	173.8
	2	61.6	4.1	2.8	9.9	3.2	49.0	−0.31	39.1	121.4
	3	60.3	4.1	3.0	7.2	3.6	41.4	−0.29	34.2	108.5
			Mean	2.7	9.0	2.9	51.3	−0.35	42.3	134.6
			SD	0.4	1.6	0.9	11.2	0.09	10.0	34.6
			CV (%)	14.8	17.8	31.0	21.8	25.7	23.6	25.7
03/09/2005	1	58.1	4.1	2.7	5.0	5.3	55.4	−0.97	50.4	286.7
	2	57.6	4.0	3.6	6.0	4.6	60.0	−0.55	54.0	192.9
	3	58.6	4.0	3.7	7.5	5.5	45.0	−0.37	37.5	132.0
			Mean	3.3	6.2	5.1	53.5	−0.63	47.3	203.9
			SD	0.6	1.3	0.5	7.7	0.31	8.7	77.9
			CV (%)	18.2	21.0	9.8	14.4	49.2	18.4	38.2

[a] Volume of water enclosed in the chamber.
[b] Duration of the incubation.

53.5 $\cdot 10^{-3}$ μmol L^{-1} h^{-1} and the mean CV for triplicate measurements was 24.1% (Table 2). These production rates are 4 to 9 times higher than the regeneration rates measured in bottles, indicating high NH_4^+ input from the sediment. The mean flux of NH_4^+ from the sediment to the water column (F_{sw}), estimated with Eq. (2), varied from 27.7 to 47.3 $\cdot$ 10^{-3} μmol L^{-1} h^{-1} (mean CV = 28.3%) (Table 2), giving a mean flux per surface unit ranging from 6.7 ± 2.6 to 13.7 ± 2.4 μmol m^{-2} h^{-1} (Fig. 2). The mean flux of NH_4^+ from the water column to the sediment (F_{ws}), calculated with Eq. (4), varied from 122.3 to 203.9 $\cdot$ 10^{-3} μmol L^{-1} h^{-1} with a mean CV of 26.7% (Table 2), corresponding to a mean flux per surface unit from 29.6 ± 3.6 to 59.2 ± 22.5 μmol m^{-2} h^{-1} (Fig. 2).

3.3. N content and ^{15}N content in the sediment

The mean N content in the sediment collected in each chamber at the end of the incubation period varied from 12.2 to 16.4 μmol N g dry sed^{-1} in the 0–0.5 cm layer and from 11.8 to 16.5 μmol N g dry sed^{-1} in the 0.5–1 cm layer (Table 3). The variation among triplicate measurements in each chamber was relatively low, with a mean CV of 4.8% and 5.9% for the 0–0.5 cm and 0.5–1 cm layers, respectively, as was the variation among chambers at each sampling station, with a mean CV of 7.3% and 7.7% for the 0–0.5 cm and 0.5–1 cm layers, respectively.

The mean ^{15}N content in excess in the sediment at the end of the incubation period ranged from 1.9 to 4.9 μmol ^{15}N g dry sed^{-1} in the 0–0.5 cm layer and from 0.6 to 1.6 μmol ^{15}N g dry sed^{-1} in the 0.5–1 cm layer (Table 3). In contrast to the N content, the ^{15}N content in excess varied considerably among the three cores sampled in each chamber (mean CV of 39.2% and 41.4% for the 0–0.5 cm and 0.5–1 cm layers, respectively) as well as among chambers at each sampling station (mean CV of 43.6% and 38.6% for the 0–0.5 cm and 0.5–1 cm layers, respectively).

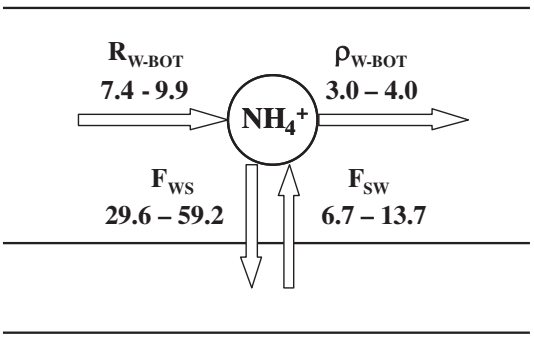

Fig. 2. Ranges of average NH_4^+ uptake and regeneration rates in bottles ($\rho_{W\text{-}BOT}$ and $R_{W\text{-}BOT}$) integrated over the water column (in μmol m^{-2} h^{-1}) and NH_4^+ fluxes per surface unit (in μmol m^{-2} h^{-1}) from the sediment to the water column (F_{sw}) and from the water column to the sediment (F_{ws}) for the three sampling stations.

Table 3
Mean (±SD, n = 3) N content and ^{15}N content in excess in the 0–0.5 and 0.5–1 cm sediment layers enclosed by each chamber at the end of the incubation period at the three sampling stations.

Date	Replicate	μmol N g dry sed^{-1}		10^{-3} μmol ^{15}N g dry sed^{-1}	
		0–0.5 cm	0.5–1 cm	0–0.5 cm	0.5–1 cm
01/09/2005	1	10.5 ± 0.5	10.9 ± 0.4	1.7 ± 0.1	0.2 ± 0.2
	2	13.4 ± 0.4	12.7 ± 1.5	1.2 ± 0.3	0.5 ± 0.4
	3	12.7 ± 1.6	11.8 ± 0.3	2.8 ± 0.7	1.2 ± 0.2
	Mean	12.2	11.8	1.9	0.6
	SD	1.5	0.9	0.8	0.5
	CV (%)	12.3	7.6	42.1	83.3
02/09/2005	1	15.7 ± 0.9	15.9 ± 1.5	2.2 ± 1.7	1.0 ± 0.4
	2	14.0 ± 0.5	14.3 ± 0.3	1.4 ± 0.9	0.8 ± 0.2
	3	16.2 ± 0.9	16.5 ± 0.6	3.7 ± 3.1	1.2 ± 0.2
	Mean	15.3	15.6	2.4	1.0
	SD	1.2	1.1	1.1	0.2
	CV (%)	7.8	7.1	45.8	20.0
03/09/2005	1	16.1 ± 0.7	14.9 ± 0.7	7.3 ± 2.0	1.8 ± 0.5
	2	16.7 ± 0.3	17.4 ± 0.5	3.8 ± 0.2	1.4 ± 0.2
	3	16.4 ± 0.3	17.3 ± 2.1	3.6 ± 1.4	1.4 ± 0.2
	Mean	16.4	16.5	4.9	1.6
	SD	0.3	1.4	2.1	0.2
	CV (%)	1.8	8.5	42.9	12.5

Table 4
Isotopic mass balance in bottle experiments: amount (μmol L^{-1}) of ^{15}N in excess added as NH_4^+ at the beginning of the experiment (^{15}N initial) and amount of ^{15}N in excess recovered in NH_4^+ and PON pools at the end of the incubation for each replicate at the three sampling stations.

Date	Experiment	^{15}N initial (μmol L^{-1})	^{15}N recovered					
			$^{15}NH_4^+$		^{15}PON		Total[a]	
			(μmol L^{-1})	(%)[b]	(μmol L^{-1})	(%)[b]	(μmol L^{-1})	(%)[b]
01/09/2005	1	0.821	0.817	99.5	0.009	1.1	0.826	100.6
	2	0.830	0.817	98.4	0.008	1.0	0.825	99.4
	3	0.825	0.809	98.1	0.009	1.1	0.818	99.2
02/09/2005	1	0.824	0.825	100.1	0.007	0.8	0.832	101.0
	2	0.848	0.824	97.2	0.008	0.9	0.832	98.1
	3	0.843	0.814	96.6	0.009	1.1	0.823	97.6
03/09/2005	1	0.847	0.839	99.1	0.008	0.9	0.847	100.0
	2	0.854	0.837	98.0	0.010	1.2	0.847	99.2
	3	0.856	0.845	98.7	0.011	1.3	0.856	100.0
	Mean	0.839	0.825	98.4	0.009	1.0	0.834	99.5
	SD	0.014	0.012	1.1	0.001	0.2	0.013	1.1

[a] Total amount of ^{15}N recovered.
[b] % of tracer initially added.

3.4. ^{15}N mass balance

Results from the ^{15}N inventories for the bottle experiments are shown in Table 4. The total amount of ^{15}N tracer in excess recovered at the end of the incubation was on average (±SD) 99.5 ± 1.1%. Almost all of the initially added tracer was accounted for in the NH_4^+ pool (98.4 ± 1.1%) whereas only 1.0 ± 0.2% was found in the particulate pool.

In the benthic chambers, 24.6 to 72.9% (mean ± SD = 59.1 ± 15.7%) of the ^{15}N initially added was recovered in the water column and the highest percentage (57.8 ± 15.9%) was observed in the dissolved pool (Table 5). The mean amount of ^{15}N tracer in excess recovered in the sediment (0–1 cm) represented 7.4 to 40.1 (mean ± SD = 16.8 ± 10.1%) of the ^{15}N in excess in the chambers at the beginning of the incubation depending on the experiment (Table 5). The total amount of ^{15}N tracer in excess recovered in the chambers, taking into account both the water column and the sediment, ranged from 61.6 to 89.9% (mean ± SD = 75.9 ± 10.0%) (Table 5).

4. Discussion

Ammonium fluxes between the benthic and pelagic compartments have often been measured (reviews by Bronk and Steinberg, 2008 and Joye and Anderson, 2008), but the techniques used generally only provide the net fluxes of NH_4^+. Our method distinguishes the influx from the efflux of NH_4^+ across the sediment–water interface by measuring the uptake and production of NH_4^+ during a single in situ incubation, using the ^{15}N tracer technique in a sample of water enclosed in a benthic chamber placed over the sediment and in a subsample thereof concomitantly incubated in a bottle.

Results from ^{15}N experiments showed that NH_4^+ uptake rates measured in chambers were not significantly different from those measured in bottles (Table 2). The reproducibility of the NH_4^+ uptake rates in benthic chambers was also comparable to that obtained for uptake and regeneration rates in bottle incubations (Table 2). The similarity of uptake rates measured in the bottle and chamber experiments indicates that incubation under benthic chambers is appropriate for

Table 5
Isotopic mass balance in benthic chamber experiments: amount (μmol) of ^{15}N in excess added as NH_4^+ at the beginning of the experiment (^{15}N initial) and amount of ^{15}N in excess recovered in NH_4^+ and PON pools and in the sediment at the end of the incubation period for each replicate at the three sampling stations.

Date	Replicate	^{15}N initial (μmol)	^{15}N recovered							
			$^{15}NH_4^+$		^{15}PON		^{15}SED[a]		Total[b]	
			(μmol)	(%)[c]	(μmol)	(%)[c]	(μmol)	(%)[c]	(μmol)	(%)[c]
01/09/2005	1	50.50	34.50	68.3	0.40	0.8	4.05 ± 0.72	8.0 ± 1.4	38.95	77.1
	2	50.78	33.88	66.7	0.51	1.0	3.74 ± 0.67	7.4 ± 1.3	38.13	75.1
	3	50.81	36.51	71.9	0.51	1.0	8.65 ± 1.15	17.0 ± 2.3	45.67	89.9
02/09/2005	1	46.97	22.90	48.8	0.36	0.8	5.66 ± 4.07	12.1 ± 8.7	28.92	61.6
	2	48.85	31.34	64.2	0.59	1.2	4.35 ± 2.63	8.9 ± 5.4	36.28	74.3
	3	51.43	35.43	68.9	0.65	1.3	9.27 ± 4.95	18.0 ± 9.6	45.35	88.2
03/09/2005	1	41.56	9.47	22.8	0.74	1.8	16.68 ± 4.38	40.1 ± 10.5	26.89	64.7
	2	45.29	20.99	46.3	0.73	1.6	9.38 ± 1.16	20.7 ± 2.6	31.10	68.7
	3	49.80	31.22	62.7	0.93	1.9	9.53 ± 5.06	19.1 ± 10.2	41.68	83.7
	Mean	48.44	28.47	57.8	0.60	1.3	7.92	16.8	37.00	75.9
	SD	3.27	8.96	15.9	0.18	0.4	4.09	10.1	6.84	10.0

[a] Mean (±SD, n = 3) amount of ^{15}N tracer recovered in the 0–1 cm surface layer of the sediment.
[b] Total amount of ^{15}N recovered.
[c] % of tracer initially added.

measuring N fluxes in the water column with the ^{15}N tracer method. The total NH_4^+ production rates determined in chambers at each sampling station and, consequently, the calculated fluxes between the sediment and the water column generally showed higher CVs than those obtained for the uptake and regeneration rates (Table 2). NH_4^+ production rates also showed relatively large differences between sampling stations compared to NH_4^+ uptake and regeneration rates, suggesting that the variation observed at each station probably resulted from variability in fluxes at the sediment–water interface rather than low measurement reproducibility. This variability was confirmed for the influx of NH_4^+ by the variability in the amount of ^{15}N recovered in the sediment at the end of each incubation period, which varied among stations, among chambers at each sampling station and even within each chamber as shown by the high SD values of the triplicate measurements (Table 3). These variations in fluxes involving exchanges with the sediment are not surprising and probably reflect the heterogeneity of the biological processes in the benthic compartment.

We assessed our data for ^{15}N mass balance, i.e. we checked whether the amount of ^{15}N added as $^{15}NH_4^+$ at the beginning of the experiments was recovered at the end in the NH_4^+ and PON pools of the water column or in the sediment in the case of the chamber experiments. Tracer inventories showed that the ^{15}N mass balance was virtually attained in the bottle experiments, as on average 99.5% (±1.1%) of the $^{15}NH_4^+$ added at the beginning of the experiments was ultimately recovered in the NH_4^+ and PON pools (Table 4). Individual values of ^{15}N recovery were within the range of those published for ^{15}N inventories in phytoplankton uptake experiments (Bronk et al., 1994; Slawyk and Raimbault, 1995). A large percentage (75.9 ± 10.0%) of the $^{15}NH_4^+$ initially added to the benthic chambers was also recovered at the end of the incubation period (Table 5). The ^{15}N tracer was mainly retrieved in the NH_4^+ and PON pools of the water column (mean ± SD = 59.1 ± 15.7%) and the fraction not found in the water column was mainly observed in the 0–1 cm layer of the sediment (mean ± SD = 16.8 ± 10.1%). However, mass balance of ^{15}N tracer was not completely attained in the chambers because an average 24% (10.1 to 38.4%, 24.1 ± 10.0%) of the ^{15}N added remained undetected at the end of the chamber experiments (Table 5). For the bottle experiments, almost all the ^{15}N added was recovered in the NH_4^+ and PON pools at the end of the incubation period, indicating that the removal of the tracer from the water column resulted essentially from uptake processes and that losses due to, for example, DON release from PON subsequent to NH_4^+ uptake or nitrification—the usual explanations for vanishing ^{15}N in uptake experiments (Bronk et al., 1994; Slawyk and Raimbault, 1995)—were negligible. These losses were probably also negligible for the chamber experiments. Therefore, the apparent ^{15}N loss observed in chambers must be due in large part to insufficient sampling of the benthic compartment. The ^{15}N content in excess in the sediment varied greatly in each chamber depending on the core sampled (Table 3), indicating that triplicate measurements were insufficient to assess the actual transfer of $^{15}NH_4^+$ from the water column to the sediment. In some chambers, the ^{15}N mass balance would have been completely attained based on only the largest value of the three measurements. In addition, only the first centimetre of the sediment was analysed for ^{15}N content. The mean ^{15}N content in the 0.5–1 cm layer represented on average 36.9 ± 13.0% (11.8–57.1%) of that found in the 0–0.5 cm layer (Table 3), suggesting that some ^{15}N content could be located even deeper in the sediment. Accordingly, there have been reports that viable MPB may be found at depths of several centimetres, especially in sandy sediments (MacIntyre et al., 1996). Moreover, some of the ^{15}N-enriched sediment in the benthic chamber experiments could have been transferred to the benthic macrofauna that was not sampled. Additionally, some of the unrecovered ^{15}N tracer may have been partially exchanged with outside water. In permeable shelf sediments, such as sandy sediment, waves, tides and horizontal pressure gradients at the sediment surface can generate non-negligible advective replacement of porewater by supernatant waters (Huettel and Gust, 1992). An advective process may have induced the replacement of part of the ^{15}N-enriched water in the benthic chambers by external non-enriched water and explain some of the ^{15}N losses. Nevertheless, advection should be limited because the chambers were deeply inserted into the sediment (ca. 10 cm). Moreover, the study area was protected from waves by the reef flat and has a low tidal amplitude.

The NH_4^+ uptake rates measured in the back-reef zone on Reunion Island fall in the range of values reported for oligotrophic environment with comparably low N concentrations (see the review by Mulholland and Lomas, 2008). The rates of NH_4^+ regeneration in the water column are also close to those obtained by Hopkinson et al. (1987, 1991) in similar coral reef environments and to those generally measured in oligotrophic conditions (for review, see Bronk and Steinberg, 2008). Both uptake and regeneration rates of NH_4^+ were relatively similar at the three sampling stations, indicating some consistency in the pelagic compartment of the study area.

The fluxes of NH_4^+ from the water column to the sediment (29.6 to 59.2 μmol m^{-2} h^{-1}) are in the upper part of the range of values generally measured in the light in comparable shallow sandy littoral zones (Eyre and Ferguson, 2002; Ferguson et al., 2004; Joye and Anderson, 2008; Reay et al., 1995; Sundbäck et al., 2000). The influx of NH_4^+ into the sediment must be due, for the most part, to uptake by benthic microalgae. Accordingly, MPB has a relatively large biomass in the back-reef zone on Reunion Island (Taddei et al., 2008). Heterotrophic bacteria can also contribute significantly to NH_4^+ uptake in the sediment (Veuger et al., 2005), but uptake by bacteria is probably very low compared to uptake by MPB, which is enhanced in the well-lit sediments of the study area. At the three stations, the mean flux of NH_4^+ from the water column to the sediment was much higher than the mean NH_4^+ uptake rate in the water (Fig. 2), which suggests that MPB is responsible for most of the NH_4^+ removal from the water column. The important role of MPB in controlling the DIN flux at the sediment–water interface has been demonstrated by numerous studies in shallow-water sediments (e.g. Joye and Anderson, 2008 and references therein). The activity of MPB in well-lit conditions has been shown to reduce the efflux of NH_4^+ from the sediment or to lead to an influx from the water column to the sediment, as observed in our study. However, the values reported in the literature do not accurately represent the actual NH_4^+ flux into the sediment because, conventional methods, based on changes in concentration, provide only the net fluxes of NH_4^+. In contrast to the method proposed here, conventional methods cannot distinguish NH_4^+ removal from the water column and its release from the sediment. The efflux of NH_4^+ is likely to continue in the light as observed in our study; the fluxes measured by the conventional methods therefore, in most cases, probably underestimate the influx into the sediment. In our study, NH_4^+ influx would have been 16 to 32% lower if the efflux had been subtracted. On the other hand, our values may be overestimated due to the addition of the ^{15}N tracer, which increases the ambient level of NH_4^+ in the benthic chambers. Furthermore, the uptake of NH_4^+ by MPB has been shown to increase with an increase in concentrations (Clavier et al., 2005; Sakamaki et al., 2006). In particular, this increase in uptake may occur if sediment N sources are insufficient to meet MPB growth demands and if MPB growth is N-limited. Such N limitation was recently highlighted for benthic diatoms in coastal Georgia (Porubsky et al., 2008) and could also be the case for MPB in sandy sediments of the back-reef zone on Reunion Island, which is poor in organic matter and probably has reduced N sources (see below).

The fluxes of NH_4^+ from the sediment to the water column measured at the different stations (6.7 to 13.7 μmol m^{-2} h^{-1}) were relatively low compared to those generally reported for shallow estuarine and coastal marine sediments (see the review by Bronk and Steinberg, 2008). However, the release of NH_4^+ from the sediment is generally measured in the dark to minimise light-dependent uptake. In our experiments, which were performed in the light, a fraction of the transferable NH_4^+ was probably captured by benthic microalgae. This may explain, at least partially, the relatively low efflux rates observed. These low rates may also

be related to the fact that the sediment of the study area is poor in organic matter (Taddei et al., 2008), which is confirmed by the low N content found at the three sampling stations (Table 3). The mean efflux of NH_4^+ at each station, despite its relatively low level, was comparable to the regeneration rates in the water (Fig. 2), suggesting that the sediment may constitute a significant N source for phytoplankton in the back-reef zone on Reunion Island.

5. Conclusion

Using the approach we described here that combines the ^{15}N tracer method and benthic chamber technique, we were able to quantify simultaneously, from a single in situ incubation, the influx and efflux of NH_4^+ at the sediment–water interface and the uptake and regeneration rates of NH_4^+ in the water column. The results obtained in a back-reef zone on Reunion Island show that the method is sufficiently sensitive for organic-poor, sandy sediments and oligotrophic waters where NH_4^+ fluxes are expected to be low. The distinction between the removal and the release of NH_4^+ from the sediment is a real improvement compared to conventional methods which only provide a net flux. The use of our combined approach in benthic studies should significantly enhance the understanding of the coupling between the benthic and pelagic compartments. This combined approach will be useful for future studies of the exchanges of other N compounds, such as nitrate or urea, which can also be extracted from seawater for isotopic analyses.

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